Carbohydrate Research, 195 (1989) 87–99
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STRUCTURAL FEATURES OF THE CELL-WALL POLYSACCHARIDES OF THE PARCHMENT LAYERS OF THE PODS OF MATURE RUNNER BEANS

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(Received March 2nd, 1989; accepted for publication, June 14th, 1989)

ABSTRACT

Cell-wall material from parchment layers of mature runner-bean pods was delignified and the holocellulose was extracted in sequence with hot water, methyl sulphoxide at 20°, and 0.5M, M, and 4M KOH at 20°, to yield the α-cellulose. The hemicelluloses solubilised by hot water and 0.5M KOH were fractionally precipitated with alcohol, and some of the fractions were resolved further by anion-exchange chromatography. The acidic oligosaccharides released on partial acid hydrolysis of two of the xylans were reduced with $NaBD_4$, methylated with deuterated iodomethane, and identified by g.l.c.–m.s. The polymers soluble in methyl sulfoxide, M KOH, and 4M KOH, selected fractions from the graded precipitation with alcohol, and some fractions from anion-exchange chromatography were subjected to methylation analysis. The major hemicellulosic polymers were glucurono- and 4-*O*-Me-glucurono-xylans with d.p. 90–200 and a ratio of Glc*p*A to 4-Me-Glc*p*A of ~1:5.4. The cell-wall xylans are acetylated, and 7 out of 10 xylose residues of the methyl sulfoxide-soluble xylan are acetylated. Evidence was obtained for the occurrence of small amounts of pectic polysaccharide, possibly in covalent association with some acidic xylans (d.p. ~30) and lignin.

INTRODUCTION

In order to understand the chemistry of dietary fibre, the composition and structure of the cell-wall polymers of various edible plant organs have been studied[1,2]. Most of our detailed studies have been concerned with parenchymatous[3,4] or immature tissues[5,6] which have undergone little or no lignification.

During maturation in the field, the pods of runner beans develop unpleasant spiky "strings" and "parchment layers" that cannot be softened by cooking and are due to secondary thickening of vascular bundles and certain specialised cells. Although the cell walls of lignified tissues of trees[7,8] and grasses[9] have been studied, little information on the cell walls of lignified tissues of vegetables is available. We

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now report on cell-wall polymers of parchment layers of the pods of mature runner beans.

EXPERIMENTAL

Preparation of cell-wall material (CWM). — Pods of mature runner beans (*Phaseolus coccineus* var. Streamline, on average 30 × 2 cm), collected from plants grown in experimental plots near the laboratory, were split lengthwise, and the inner parenchyma and outer chlorenchyma tissues were removed by scraping obliquely to expose the parchment layers. The exposed parchment layers were blended gently in distilled water in order to remove any loosely adhering soft tissues, which were removed by decantation. The parchment layers were then cut into small pieces, vigorously blended in distilled water for 10 min, and allowed to settle. The floating fine particles were decanted and the sediment was ball-milled in distilled water at 2° for 3 days in order to finely sub-divide the fibres. The CWM was isolated by centrifugation and washed once with distilled water.

Extraction of pectin and delignification of CWM. — When the CWM was extracted with aq. 0.6% oxalate at 95° for 1.5 h, only a small proportion of the pectic material was solubilised. The residue was delignified by treatment[10] with sodium chlorite–HOAc at 70° for 4 h, then washed thoroughly with cold distilled water, and the polysaccharides were extracted from the holocellulose.

Determination of lignin. — The Klason lignin was removed by dispersing a sample of the CWM in (aq.) 72% H_2SO_4, diluting to M acid, and heating at 100° for 4 h.

Determination of acetyl. — The method of McComb and McCready[11] was applied to the CWM and methyl sulfoxide-soluble xylan.

Extraction of the holocellulose. — The holocellulose (3.5 g dry weight) was extracted in sequence with (*1*) hot water (150 mL, pH 5.5) at 80° for 1.5 h, (*2*) methyl sulfoxide (75 mL, residue was dispersed initially by ultrasonication) at 20° for 12 h, (*3*) 0.5M, M, and 4M KOH (50 mL of each containing 20mM $NaBH_4$) under argon at 20° for 2 h. Each extract, obtained by centrifugation, was filtered, dialysed, and freeze-dried. The alkaline extracts were acidified to pH 5 with HOAc before dialysis. The α-cellulose residue was dialysed and an aliquot was freeze-dried for analysis. The absorption spectra of the cell-wall polymers were determined after dispersion in water (hot water and methyl sulfoxide soluble) or 0.1M NaOH (alkali-soluble fractions).

Graded precipitation with alcohol. — The concentration of ethanol was increased in steps of 20%. Each mixture was placed in an ice bath for 1 h, and the precipitate was collected by centrifugation and freeze-dried.

Ion-exchange chromatography. — Some of the ethanol-precipitated fractions were stirred overnight in 0.05M phosphate buffer (1 mL, pH 6.5), insoluble material was removed by centrifugation, and the supernatant solution was added to a column (7 × 1 cm) of DEAE-Trisacryl (PO_4^{3-} form) and eluted at 16 mL/h with

0.05M phosphate buffer (40 mL) then with a linear gradient (100 mL) of NaCl (0→1M in phosphate buffer). The absorption of fractions (2 mL) at 280 nm was monitored and aliquots (20 μL) were assayed for total carbohydrate using the phenol–sulphuric acid method[12]. The appropriate fractions were combined, dialysed, and freeeze-dried.

Partial acid hydrolysis. — The 4M KOH-soluble polysaccharides and the water-insoluble material from the 0.5M KOH extract (~20 mg) were hydrolysed in 0.2M trifluoroacetic acid at 100° for 2 h. Excess of acid was evaporated with water under vacuum. The residual oligosaccharides were reduced with $NaBD_4$, methylated (CD_3I), and examined by g.l.c. (1-m OV-1 column) and g.l.c.–m.s. (e.i. mode)[3].

Monosaccharide analysis. — Neutral sugars were released by modified Saeman hydrolysis and analysed as their alditol acetates by g.l.c.[13]. Uronic acid was determined by a modification[14] of the colorimetric method of Blumenkrantz and Asboe-Hansen[15].

Methylation analysis. — The method of Hakomori was used[16,17]. Any insoluble material after the first methylation was removed by centrifugation and remethylated. One fraction was reduced with $LiAlD_4$ after methylation[6] in order to determine the type and amount of uronic acid present. Each methylated fraction was hydrolysed in aq. 90% formic acid (1 mL) at 100° for 2 h followed by 0.25M H_2SO_4 (1 mL) at 100° for 12 h. The products were converted into their alditol acetate derivatives and analysed by g.l.c. and g.l.c.–m.s. (OV-225 column). G.l.c.–m.s. was performed on a Kratos MS9/50 mass spectrometer and the mass spectra were identified using the data of Jansson *et al.*[18]. The values for the partially methylated alditol acetates were corrected by using the molar response factors recorded by Sweet *et al.*[19].

RESULTS AND DISCUSSION

Fractionation of CWM. — The CWM of parchment layers was heavily lignified and contained 20% of Klason lignin; hence, only a small amount of the "pectic material" could be solubilised by extraction with oxalate. The delignified residue was extracted in sequence with a range of solvents and the data on the fractions are given in Table I.

The delignification treatments solubilised significant amounts of cell-wall polymers, but their carbohydrate contents were low. Hot water solubilised a significant amount of polysaccharides rich in xylose from the holocellulose and subsequent treatment with methyl sulfoxide solubilised a small amount of xylans. The bulk of the acidic xylans were solubilised by 0.5M KOH, presumably by hydrolysing ester links between acidic xylans and oxidised (degraded) lignin. The hemicelluloses solubilised by M and 4M KOH contained significantly higher levels of xylans, and the average ratio of xylose to uronic acid of 19:1 suggested that they contained mainly acidic xylans. The final residue was mostly cellulose, unlike the

TABLE I

CARBOHYDRATE COMPOSITION OF THE FRACTIONS OBTAINED BY SEQUENTIAL EXTRACTION OF CWM

Fraction	*Yield (mg/g)*	*"Anhydro sugar" (μg/mg dry wt.)*							
		Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total*
CWM		4	5	288	4	4	386	77	769
Oxalate	1.7								
Chlorite/HOAc (2 h)	34.2	4	13	66	9	4	18	39	153
Chlorite/HOAc (4 h)	8.5	4	6	117	18	13	71	25	254
Holocellulose	705.5	5	6	322	3	5	457	46	844
Hot water	41.4	9	9	455	2	17	7	79	578
$(CH_3)_2SO$	11.3	6	11	560	2	9	15	61	663
0.5M KOH	97.8	6	16	510	2	14	8	113	668
M KOH	31.2	2	6	771	1	2	7	59	848
4M KOH	31.6	2	11	759	2	2	8	51	835
α-Cellulose	223.1	2	3	36	5	3	868	35	952

TABLE II

CARBOHYDRATE COMPOSITION OF THE HOT WATER SOLUBLE POLYMERS PRECIPITATED WITH ETHANOL

Fraction	*Yield* (%)	*"Anhydro sugar" (μg/mg dry wt.)*							
		Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total*
No EtOH, insoluble residue	1.8	46	186	203	23	20	84	117	679
EtOH, 0–20%	6.1[a]	3	64	345	23	5	87	65	592
20–40%	5.7	3	94	42	22	7	22	114	304
40–60%	25.6	5	223	239	9	10	16	90	592
60–80%	37.2	15	31	601	4	9	23	96	778
80% supernatant	25.4	11	130	201	12	8	24	96	482
Major fractions from 60–80% precipitate									
(Recovery from column)	89.4								
1–17	68.2[b]	18	25	387	15	20	20	46	531
18–25	27.3	20	30	180	12	12	55	73	382

[a]Yield given as % of EtOH-soluble material. [b]Yield given as % of material recovered from the column.

TABLE III

CARBOHYDRATE COMPOSITION OF THE 0.5M KOH-SOLUBLE POLYMERS PRECIPITATED WITH ETHANOL

Fraction	*Yield (%)*	*"Anhydro sugar" (μg/mg dry wt.)*							
		Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total*
No EtOH, insoluble residue	58	5	8	585	1	27		79	705
EtOH, 0–20%	15.4[a]	3	34	66	5	21	32	320	481
20–60%	30.8	7	32	414	2	25	25	106	611
60–80%	20	14	61	144	6	55	11	106	397
80% supernatant	33.8	12	35	50	7	16	30	90	240
Major fractions from 20–60% precipitate									
(Recovery from column)	93.6								
1–10	38.9[b]	10	35	555	6	6	24	130	766
26–39	41.2	12	60	314	5	41	36	118	586
80% supernatant									
(Recovery from column)	74.8								
1–11	20.6[b]	20	30	35	11	7	20	4	127
26–45	36.4	7	22	8	8	1	17	15	77
46–69	21.5	5	23	3	6	Tr[c]	17	10	64

[a]Yield given as % of EtOH-soluble material. [b]Yield given as % of material recovered from the column. [c]Trace.

corresponding residues from cell walls of soft tissues[3,4,6], which contain significant amounts of highly branched pectic polysaccharides. The proportion of uronic acid in the various fractions ranged from 3–20%. The uronic acid contents of the polymers soluble in hot water, methyl sulfoxide, and 0.5M KOH were significantly higher than those detected in the polymers soluble in M and 4M KOH and in the fractions from the CWM of beeswing wheat bran[20], which suggested the presence of pectic polysaccharides. The total carbohydrate content of the polymers soluble in hot water, methyl sulfoxide, and 0.5M KOH suggested that they contained significant amounts of non-carbohydrate material, probably of phenolic origin since aqueous solutions of the polymers at pH 6.5 had λ_{max} 280 nm.

Graded precipitation with alcohol. — The polymers soluble in hot water and 0.5M KOH were subjected to a graded precipitation with ethanol prior to anion-exchange chromatography of some of the fractions. The polymers soluble in M and 4M KOH were not soluble in the buffer tested, even at pH 11, and were not fractionated further. Tables II and III show the yields and carbohydrate composition of the various fractions. Most of the fractions were rich in xylose and contained significant amounts of rhamnose, arabinose, and galactose, which are usually present in pectic polysaccharides. From the total carbohydrate content of the fractions, it was inferred that they contained significant amounts of non-carbohydrate material, probably of phenolic origin.

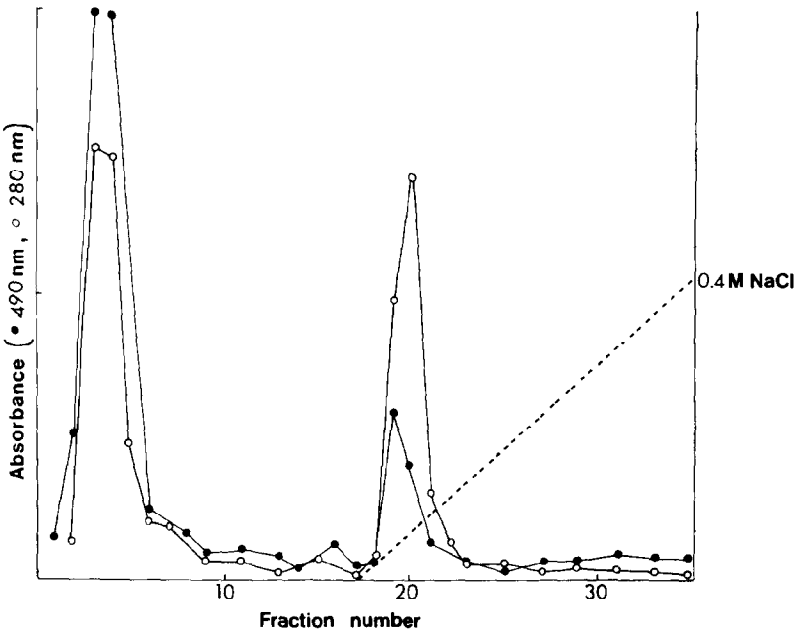

Fig. 1. Fractionation on DEAE-Trisacryl of the 60–80% EtOH ppt. from the hot water-soluble polymers: —●—, total carbohydrate; —○—, absorbance at 280nm; ----, solvent gradient. For details, see text.

TABLE IV

PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM HEMICELLULOSIC FRACTIONS OF PARCHMENT CELL-WALL MATERIAL

Alditol	*Relative mol (%)*											
	HW-soluble[a]		$(CH_3)_2SO$-soluble	*0.5M KOH-soluble*						1M *KOH-soluble*	*4M KOH-soluble*	
	1–17	*18–25*		*20% EtOH ppt.*	*20% EtOH ppt.*[b]	*60% EtOH ppt.*[c]	*80% EtOH ppt.*	*80% EtOH soluble*	*Insoluble residue*		$CHCl_3$-soluble	$CHCl_3$-insoluble
3,4-Me_2-Rha	0.3	3.5	0.3	1.5	0.9	0.7	2.1	3.1				
2,4-Me_2-Rha	1.2	3.7	0.9	0.6	0.3	1.4	1.4	2.3	0.6			
2-Me-Rha	Tr[d]											
4-Me-Rha		3.1				0.6		2.9				
3-Me-Rha	0.2	Tr		0.4	0.2		0.9					
2,3,5-Me_3-Ara	1.3	3.2	1.0	3.5	1.9		13.5	8.8	0.3	0.2	0.3	
3,5-Me_2-Ara	0.4	Tr	0.3	2.1	1.5		7.0	6.9				
2,5-Me_2-Ara			0.1	2.5	1.0		1.6	3.5		0.3		
2,3-Me_2-Ara	1.4	1.8	0.4	2.4	1.6	0.5	4.7	2.2	0.2	0.2		
2,3,4-Me_3-Xyl	0.9	1.5	1.0	1.7	1.5	0.6	1.0	1.3	0.6	0.5	0.5	
2,3-Me_2-Xyl	82.4	52.1	84.1	59.8	40.5	88.1	41.3	31.6	92.6	93.6	93.5	84.7
3-Me-Xyl	5.9	4.5	5.6	6.2	6.2	2.0	6.0	1.5	4.0	1.9	1.7	7.5
2-Me-Xyl		1.1	1.9			1.5				Tr	Tr	Tr

2,3,6-Me_3-Man		0.9									0.6	
3,4-Me_2-Man		3.4										
2,4-Me_2-Man	0.6											
2,3,4,6-Me_4-Gal							Tr					
2,3,6-Me_3-Gal	3.1	2.2	2.0	6.2	5.5	2.2	9.6	4.2	0.9	Tr	Tr	Tr
2,4,6-Me_3-Gal	0.2	Tr					1.3				Tr	
2,3,4-Me_3-Gal	0.4				1.1[e]		2.0	2.4				
2,3-Me_2-Gal					28.2[e]		0.7					
2,4-Me_2-Gal					2.6[e]		3.6	2.5				
3-Me-Gal					1.2[e]							
2,3,4,6-Me_4-Glc		3.5	0.1	0.6		0.4		5.8				
2,3,6-Me_3-Glc		8.3	1.4	4.0	3.0	0.6	1.4	9.0	0.7		1.8	
3,4,6-Me_3-Glc		1.2		1.3	0.3		1.3	3.6				
2,4,6-Me_3-Glc	0.4	1.2		2.5	0.9		0.3	2.4				
2,6-Me_2-Glc		0.8										
2,4-Me_2-Glc							0.3	0.7				
3,4-Me_2-Glc								2.7				
2,3-Me_2-Glc											1.2	
3-Me-Glc	0.4											
Hexitols	0.9	4.1	1.3	2.6	1.7	1.3	0.3	2.7	Tr	3.2	0.5	7.8
D.p.[f]	98	39	92	39	31	152	47	25	161	191	190	

[a]60–80% EtOH ppt. [b]Carboxyl-reduced. [c]Fractions 1–10. [d]Trace. [e]Deuterium labelled. [f]Degree of polymerisation of acidic xylans.

Anion-exchange chromatography. — Three fractions, namely the 60–80% ethanol precipitate from the hot-water-soluble polymers, the 20–60% precipitate from the 0.5M KOH-soluble polymers, and the polymers in the 80% supernatant solution, were fractionated on DEAE-Trisacryl M. The recoveries from the columns were in the range 75–94% (Tables II and III). The carbohydrate elution profiles of these fractions were similar and each of the "carbohydrate peaks" had u.v. absorption (see Fig. 1). Xylose was the preponderant sugar and these fractions probably arose from lignin–carbohydrate complexes in the middle lamella region. For the 80% EtOH supernatant solution from the polymers soluble in 0.5M KOH, significantly larger amounts of u.v.-absorbing material were associated with the "carbohydrate peaks", and this is reflected in the relatively low recoveries of sugars in the separated fractions. These fractions were also probably derived from lignin–carbohydrate complexes. Lignin–carbohydrate complexes have been isolated from several plant sources[21–23].

Methylation analysis. — The polymers soluble in methyl sulfoxide, M KOH, and 4M KOH, selected fractions from the graded precipitation with alcohol, and some fractions from anion-exchange chromatography were subjected to methylation analysis (Table IV). The derivatives from 3-Me-Xyl, 2-Me-Xyl, and 2,3,6-Me_3-Gal co-chromatographed, and their "relative" amounts were calculated by mass spectrometry from the relative abundance of the ions at *m/z* 190 and 130 (3-Me-Xyl), 261 and 118 (2-Me-Xyl), and 233 and 118 (2,3,6-Me_3-Gal). The polymers soluble in 4M KOH gave a significant amount of methylated material that was insoluble in chloroform–methanol, which was remethylated and re-analysed. With the exception of this insoluble material, each of the fractions examined gave <5% of hexitol hexa-acetates (Table IV). The methylated material from the 20% EtOH precipitate from the polymers soluble in 0.5M KOH was carboxyl-reduced ($LiAlD_4$) then analysed. Most of the deuterium label was incorporated into the 2,3-Me_2-Gal derivative, which would have arisen from (1→4)-linked Gal*p*A residues of pectic polysaccharides. The Glc-*6,6-*d_2 derivative from terminal Glc*p*A and 4-Me-Glc*p*A was not detected, presumably because these uronic acids were present in very small amounts. However, their occurrence in the acidic xylans was confirmed by characterising the acidic oligosaccharides released on partial acid hydrolysis (see below). Approximate estimates of the d.p. of the xylans, obtained from the ratios of (1→4)- + (1→2,4)- + (1→3,4)-linked xylose residues to terminal xylose residues, are included in Table IV.

The carbohydrate compositions given in Tables I–III and the results of methylation analysis of selected polymers (Table IV) revealed the occurrence of three main groups of polysaccharides, namely, (*a*) slightly substituted xylans having d.p. 190 (Table IV, columns 10 and 11) and 150 (Table IV, columns 6 and 9); (*b*) moderately substituted xylans (d.p. 90–100) closely associated with small amounts of pectic polysaccharides and degraded phenolics (Table IV, columns 1 and 3); and (*c*) relatively, highly substituted xylans in association with significant amounts of pectic polysaccharides and degraded lignin (Table IV, columns 2, 4, 5, 7, and 8).

The presence of pectic polysaccharides in the above fractions was inferred from the occurrence of variously linked rhamnose residues, (1→5)-linked Ara*f* residues, and the detection of Gal-d_2 derivatives in the 20% EtOH precipitate from the polymers soluble in 0.5M KOH (Table IV, column 5). These results are good evidence for the occurrence of pectic polysaccharides in the cell walls of heavily lignified tissues of dicotyledons.

The parchment layers contained 79 μg of acetyl/mg of CWM, which indicates the molar ratio of acetyl to xylose to be 1:1.2. The molar ratio of acetyl to xylose in the polymers soluble in methyl sulfoxide was 1:1.4, which suggests that 7 out of 10 xylose residues in the xylan were acetylated.

Partial acid hydrolysis. — The acidic oligosaccharides released on partial acid hydrolysis of the acidic xylans solubilised by 0.5M (water-insoluble residue, Table III) and 4M KOH were reduced with $NaBD_4$, methylated (CD_3I), and examined by g.l.c. on OV-1[3,24]. The use of CD_3I enables the terminal Glc*p*A and terminal 4-*O*-Me-Glc*p*A to be distinguished and their relative proportions to be determined. The oligosaccharide deriatives were characterised by (*a*) retention time (*T*, relative to that of methylated cellobi-itol) and (*b*) the diagnostic ions in the mass spectra. The application of established principles in order to obtain information on sequence and the nomenclature for the degradation of methylated oligosaccharide-alditols have been discussed[3,24].

The oligosaccharide derivatives from both xylans gave four main peaks. The first peak was non-carbohydrate in origin, and the remaining peaks (peaks 1–3) were obtained in the ratios 2:3:1 and 2:3:3 for the xylans soluble in 0.5M and 4M KOH, respectively. Since similar oligosaccharide derivatives were detected in both chromatograms, the identity of the oligosaccharide derivatives detected in the first two peaks from the polymer soluble in 0.5M KOH will be described. The relative abundance of the fragment ions from the oligosaccharide derivatives in peak 3 were too small to allow unambiguous identification. However, the occurrence of tetrasaccharide derivatives containing terminal Glc*p*A and 4-Me-Glc*p*A could be inferred.

Peak 1 (max *T* 1.25) was eluted in the region for a methylated disaccharide-alditol and gave ions of the aA series [*m/z* 245 (0.7%), 210 (11.0%), 175 (5.5%), 242 (3.8%), 207 (60.7%), and 172 (3.0%)] which showed the presence of non-reducing, terminal glucuronosyl and 4-Me-Glc*p*A groups. Intense ions at *m/z* 95 (39.1%), 142 (8.5%), and 204 (aldJ_2, 13.2%) demonstrated a 2-substituted pentitol derivative. The above data identify the parent oligosaccharides as Glc*p*A-(1→2)-Xyl*p* and 4-Me-Glc*p*A-(1→2)-Xyl*p*. From the ratio of the relative abundance of the ions at *m/z* 245 and 242, as well as 210 and 207, the ratio of Glc*p*A to 4-Me-Glc*p*A was inferred to be 1:5.5. The ions at *m/z* 175 and 172 can also arise by fragmentation of residues other than Glc*p*A and cannot be used to obtain the ratio of uronic acids.

Peak 2 (max *T* 2.90) was eluted in the region for a methylated trisaccharide-alditol. Selected-ion monitoring with g.l.c.–m.s. (e.i. mode) for the ions at *m/z*

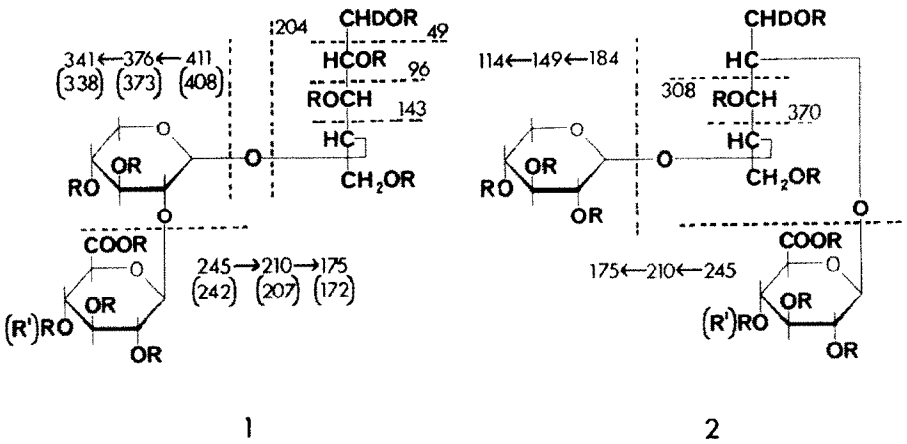

Fig. 2. Mass-spectral fragmentation patterns of the methylated oligosaccharide-alditols where R = CD_3 and R′ = CH_3; structures 1 and 2 correspond to components from peak 2. See text for details of the relative abundance of the ions.

245, 242, 210, 207, 184, and 149 revealed 2 components, of which the slower moving was present in significantly larger amount. This derivative gave ions of the aA [*m/z* 245 (2.9%), 210 (16.9%), 175 (16.2%), 242 (5.6%), 207 (90.5%), 172 (6.1%)] and baA [411 (0.1%), 376 (0.9%), 341 (2.1%), 408 (0.2%), 373 (10.7%), 338 (0.5%)] series which, in conjunction with the derivatives detected in peak 1, identified the sequence Glc*p*A(and 4-Me-Glc*p*A)-(1→2)-Xyl*p*. Ions at *m/z* 96 (52.1%), 143 (12.1%), and 204 (aldJ$_2$, 22.2%) demonstrated a 4-substituted pentitol derivative (Fig. 2, structure 1). These data and the results from methylation analysis of the parent polysaccharide (Table IV, column 9) are consistent with the structures Glc*p*A-(1→2)-Xyl*p*-(1→4)-Xyl*p* and 4-Me-Glc*p*A-(1→2)-Xyl*p*-(1→4)-Xyl*p* for the parent oligosaccharides. From the relative abundance of the ions at *m/z* 210 and 207, the ratio of Glc*p*A to 4-Me-Glc*p*A was inferred to be 1:5.4.

The smaller component (~10% of the total peak), which was eluted at the leading edge of the peak, gave the ions of the aA [*m/z* 245 (0.3%), 210 (7.3%), 175 (2.9%), 242 (1.6%), 207 (32.2%), 172 (1.3%)] and cA series [*m/z* 184 (14.5%), 149 (19.9%), 114 (7.8%)], which indicated the presence of non-reducing Glc*p*A, 4-Me-Glc*p*A, and pentosyl groups (Fig. 2, structure 2). The ions at *m/z* 370 (1.3%), 308 (0.2%), and 107 (*i.e.*, 308 − 201) (51.0%) indicated that the parent oligosaccharides were probably Xyl*p*-(1→4)-[Glc*p*A-(1→2)]-Xyl*p*- and its 4-Me-Glc*p*A analogue.

It is concluded that (*a*) the CWM of parchment layers of the pods of runner beans contain mainly cellulose, acidic xylans, and lignin(s), together with small amounts of pectic polysaccharides possibly in covalent association with some acidic xylans (d.p. ~30) and lignin; these results support the histochemical observations that lignification is initiated in the middle lamella region; (*b*) the cell-wall xylans are acetylated, and 7 out of 10 xylose residues of the xylan soluble in methyl sulfoxide are acetylated; (*c*) the acidic xylans are heterogeneous and the major

polymers have d.p. in the range 90–200. The ratio of Glc*p*A to 4-Me-Glc*p*A in the acidic xylans was ~1:5.4. In the major acidic xylan from the 0.5M KOH extract, ~1 in 10 of the xylose residues carried a uronic acid substituent, whereas about 1 in 20 of the xylose residues in the 4M KOH-soluble xylan carried a uronic acid substituent.

ACKNOWLEDGMENTS

We thank John Eagles and Keith Parsley for the mass spectrometry, and Barry Stevens for helpful discussions.

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